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Research Paper :

The root part of *Costus specious* possesses *in vitro* cytotoxic potential against human cancer cell lines from colon, liver and prostate origin

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ABSTRACT

The aim of this research work was to evaluate the anticancer potential of the extracts of the traditional medicinal plant *i.e. Costus speciosus*. The extracts of the root part were screened for *in vitro* cytotoxicity by means of SRB assay on five human cancer cell lines : colon cancer cells (COLO-205, HT-29, SW-620), liver cancer cells (HEP-2) and prostate cancer cells (DU-145). The assay yielded very interesting and significant consensus from medicinal view point as all the extracts of the plant showed remarkable cytotoxic effect on each human cancer cell line in the range of 70-92%.

Key words : *Costus speciosus*, Human cancer cells, *In vitro* cytotoxicity, SRB-assay

Costus speciosus (Koenig ex Retz.) J.E.Smith, commonly known as Keukand belonging to the Zingiberaceae family, is a succulent herb with long leaf, white flowers and fruits, globose or ovoid capsules. The plant is native to many Pacific Islands and is found throughout India in moist localities.

Six compounds were isolated from the rhizome of *Costus speciosus* and elucidated as diosgenin(1), prosapogenin B of dioscin (2), diosgenone(3), cycloartanol (4), 25-en-cycloartenol(5) and octacosanoic acid (6) of which compounds 3-6 were obtained from *Costus speciosus* for the first time (Qiao *et al*., 2002). Rhizome of the plant possesses antifungal principle (Bandara *et al*., 1988) and hypoglycemic effects (Mosihuzzaman *et al*., 1994). Different parts of the plant possess polyphenol content and antioxidant activity (Vijayalakshmi and Sarada, 2008). The root extract of *Costus speciosus* possesses antihyperglycemic, antihyperlipemic and antioxidative effects, which may prove to be of clinical importance in the management of diabetes and its complications as hyperglycemia, abnormal lipid and antioxidant profiles are the most usual complications in diabetes mellitus (Bavarva and Narasimhacharya, 2008). In the present study, *in vitro* cytotoxicity of the root extracts (ethanolic, 50% ethanolic, aqueous) of the plant with appropriate positive controls has been carried out against five human cancer cell lines from three different origins.

MATERIALS AND METHODS

Plant material:

The plant was collected from Pounichak village of district Jammu J&K, India in the month of March.The freshly collected plant was chopped, shade dried and ground into powder. Powdered dried seed material was then extracted with different solvents at room temperature to obtain extracts for bioevaluation.

Preparation of plant extracts:

For the ethanolic extract, dried ground plant material (100g) was percolated with 95% ethanol, then concentrated to dryness under reduced pressure. For aqueous ethanolic extract, another lot of dried ground plant material (100g) was percolated with 50% ethanol and concentrated to dryness under reduced pressure. The hot water extract was obtained by boiling dried ground plant material (100g) for 30 min in distilled water (300ml). The ethanolic extract was dissolved in Dimethyl sulfoxide (DMSO), the aqueous ethanolic extract in 50% DMSO and the water extract in sterile water to form stock solutions 20mg/ml. The microbial contamination was controlled by addition of 1% gentamycin in complete growth medium *i.e.* used for dilution of stock solutions to prepare working test solutions 200µg/ml. All extracts were freeze dried.

Preparation of positive controls:

Positive controls, *viz.,* Mitomycin-C and 5-Flurouracil were prepared in distilled water, then diluted in gentamycin

medium to obtain desired concentrations of 2 x 10^{-5}M and 2 x 10^{-5}M.

Human cancer cell lines:

The human cancer cell lines were obtained either from National Center for Cell Science, Pune, India and cultured in RPMI-1640 medium (pH 7.4), supplemented with Fetal Calf Serum (10%), pencillin (100 units/ml), streptomycin (100µg/mL) and glutamine (2mM).

Cytotoxic assay:

Test material (extracts) were subjected to *in vitro* anticancer activity against various human cancer cell lines (Monks *et al*.,1991). For the assay (in brief), the cells were grown in tissue culture flasks in growth medium at 37°C in an atmosphere of 5% CO_2 and 90% relative humidity in a CO_2 incubator. The cells at subconfluent stage were harvested from the flask by treatment with trypsin (0.05% trypsin in PBS containing 0.02% EDTA) and suspended in growth medium. Cells with more than 97% viability (Trypan blue exclusion) were used for determination of cytotoxicity. An aliquot of 100ìl of cells (10^5 cells/ml) was transferred to a well of 96-well tissue culture plate. The cells were allowed to grow for 24 h. Test material was then added to the wells and cells were further allowed to grow for another 48 h.

Sulphorhodamine B (SRB) assay:

The antiproliferative SRB assay was performed to assess growth inhibition which estimates cell number indirectly by staining total cellular protein with the dye SRB (Skehan *et al*.,1990).In brief, the cell growth was stopped by gently layering 50µl of 50% (ice cold) trichloroacetic acid on the top of growth medium in all the wells. The plates were incubated at 4°C for an hour to fix the cells attached to the bottom of the wells. Liquid of all the wells were then gently pipetted out and discarded. The plates were washed five times with distilled water and were air-dried. SRB 100µl (0.4% in 1% acetic acid) was added to each well and the plates were incubated at room temperature for 30 min. The unbound SRB was quickly removed by washing the cells five times with 1% acetic acid and the bound dye was dissolved in tris buffer (100µl, 0.01M, pH 10.4). Plates were gently stirred for 5min. on a mechanical stirrer and the optical density was recorded on ELSIA reader at 540nm.

Suitable blanks and positive controls were also included. Each test was done in triplicate and the values reported herein are mean values of three experiments.

Calculations:

The cell growth was determined by substracting average Absorbance (OD) value of respective blank from the average Absorbance (OD) value of experimental set. Per cent growth in the presence of test material was calculated as under

$$\frac{\text{Growth in the presence of test material}}{\text{Growth in the absence of the test material}} \times 100$$

Per cent growth inhibition in the presence of test material was calculated as under:

100 – per cent growth in the presence of test material

Criteria for activity:

The growth inhibition of 70% or above was considered active.

RESULTS AND DISCUSSION

The devastation caused by cancer is staggering to contemplate and it is considered that the management of cancer is still not upto the mark. We are in emergent need of drugs for the treatment of cancer which should be less toxic and more potent. Indian medicinal plants have assumed real significance and there is a need to screen more Indian medicinal plants for their anticancer

Table 1 : Growth inhibitory effect of the root extracts of *Costus speciosus* along with particular positive controls against human cancer cell lines

Generic name of the plant	Part used	Extract	Human cancer cell lines				
			COLO-205	HT-29	SW-620	HEP-2	DU-145
			Growth inhibition (%)				
Costus	Root	Ethanolic	85	87	86	92	89
speciosus		50% Ethanolic	81	79	83	81	81
		Hot water	74	70	70	71	70
Positive controls		Conc. (M)					
Mitomycin-C		1 x 10^{-5}	-	-	-	82	72
5-Flurouracil		1 x 10^{-5}	50	61	67	-	-

The concentration of extracts employed in each case was 100µg/ml.

The mark (-) means that the particular human cancer cell line was not treated with that particular positive control.

activity *in vitro*. Keeping in view these facts, the *in vitro* cytotoxic assay of *Costus speciosus* (a traditionally used medicinal plant) was conducted via extracts (ethanolic, 50% ethanolic, hot water) with appropriate positive controls against five human cancer cell lines namely of colon (COLO-205, HT-29, SW-620), liver (HEP-2) and prostate (DU-145). Results demonstrate that all the extracts of above mentioned medicinal plant was found active as they inhibited the growth of all the five human cancer cell lines from three different tissues. The ethanolic extract showed activity in the range of 85-92%. The growth inhibition showed by 50% ethanolic extract was in the range of 79-83%. The aqueous extract was found active in the range of 70-74%.

Conclusion:

The present investigation was carried out for the possible exploitation of the Indian medicinal plant *i.e. Costus speciosus* in the management of cancer. The active ingredient(s) from the root part of the plant will surely serve as lead molecule(s) in the development of anticancer drugs for colon, liver and prostate carcinomas to provide a great promise and service to cancer patients.

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